U.S. Patent Application
Serial No. 09/322,353

Attorney Docket No. 9855-26U1
(OTT 3038-1)

Clean Copy of Substitute Paragraphs, As Amended in the Amendment Corresponding to the Office Action Dated 30 January 2001

i) Please delete the paragraph at page 4, lines 2-3 and substitute in place thereof the paragraph amended to read as follows.

In a further aspect, the KDR^+ cells are isolated using a conjugated vascular endothelial growth factor or a molecule derived therefrom.

ii) Please delete the paragraph at page 14, lines 21-28 and substitute in place thereof the paragraph amended to read as follows.

The invention also includes a method of obtaining a cell population enriched for long-term repopulating human hematopoietic stem cells wherein KDR^+ cells are isolated using a conjugated vascular endothelial growth factor. This method simply capitalizes on the affinity of the KDR-VEGF receptor-ligand interaction to select cells expressing KDR on their surfaces by binding such cells, via the KDR present on the surface of the cell, to VEGF conjugated to, for example, a solid support matrix. Thus, the VEGF-conjugate can be used to affinity-purify the KDR expressing cells by standard methods well-known in the art.

iii) Please delete the paragraph at page 40, lines 16-18 and substitute in place thereof the paragraph amended to read as follows.

The mouse monoclonal antibody (clone 260.4), raised against the KDR soluble protein and recognizing the extracellular KDR domain, was obtained from Gesellschaft für Biologische Forschung, GBF, Braunschweig, Germany.

iv) Please delete the paragraph at page 42, lines 1-14 and substitute in place thereof the paragraph amended to read as follows.

HPCs were seeded in 0.9% methylcellulose fetal calf serum free (FCS$^-$) medium supplemented with saturating amounts of HGFs [flt3, kit ligand (FL, KL), basic fibroblast GF (bFGF) (100 ng/ml each), interleukin 6 (10 ng), IL3 (100 U), granulomonocyte colony-stimulating factor (GM-CSF) (10 ng), G-CSF (500 U), M-CSF (250 U), thrombopoietin (Tpo) (100 ng), erythropoietin (Epo) (3 U)]. CFU-Mix/BFU-E and CFU-GM colonies comprised >5 x 103 and >10^3 cells, respectively (Gabbianelli et al., 1995, Blood 86:1661-1670). A more limited HGF combination comprised IL3, GM-CSF, Epo at the indicated dosages (Gabbianelli et al., 1995, Blood 86:1661-1670) (this culture condition was also utilized for NOD-SCID mice BM mononuclear cell (MC) clonogenic assay). CFU-Mix/BFU-E and CFU-GM colonies comprised >500 and >100 cells respectively. For detection of human colonies, the colony DNA was processed for PCR using KlenTaq-1 DNA polymerase (Clontech, Palo Alto, CA) and primers recognizing human a-satellite sequences on chromosome 17 (Warburton et al., 1991, Genomics 11:324-333).